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Hierarchical structuring of genetic variation at differing geographic scales in the cultivated sugar kelp *Saccharina latissima*

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ABSTRACT

The cultivation of macroalgae for biofuels, food and fertilisers has increased dramatically in recent years. The demand for such algal-derived products means that large scale cultivation in coastal waters will become necessary to provide sufficient algal biomass. As part of the process of establishing new macroalgal farms, the potential for gene flow between cultivated specimens and natural populations needs to be taken into consideration. Consequently, in the present study we have used a combined population genetic and hydrodynamic modelling approach to determine potential levels and patterns of gene flow in the kelp *Saccharina latissima*. Microsatellite analysis of 14 populations sampled across the northern part of the Irish Sea indicated four distinct genetic clusters. These were consistent with dispersal patterns indicated by the particle tracking model and show a combination of isolation by distance and genetic structuring due to local hydrodynamic conditions. At smaller scales (less than a few 10s of km), gene flow appears to be fairly extensive, with evidence of local population connectivity due to local currents. At larger scales, however, factors such as freshwater efflux and open water would appear to represent barriers to gene flow. Together, these patterns suggest that factors other than simple geographical distance and proximity need to be taken into account when planning the siting of kelp farms with the aim of minimizing gene flow to and from natural populations.

Keywords

Algae, Cultivation, Dispersal, Gene flow, Hydrodynamic modelling, Kelp, Population genetics, *Saccharina latissima*

1. Introduction

The popularity of macroalgal cultivation is increasing in North Western Europe owing to its applicability for biofuel, food supplements and fertiliser (Wei et al., 2013). It is estimated that 2,000 – 3,000 dry tonnes (equivalent to 25,000 – 40,000 tonnes wet weight) of macroalgae is harvested from the wild per year in the United Kingdom to produce food and feed products as well as speciality chemicals and fertilisers (Schlarb-Ridley and Parker, 2013), but these figures lag far behind those from Southeast Asia, where over 20 million tonnes were produced in 2014 (FAO, 2016; Buschmann et al., 2017). The potential exploitation of macroalgae in a range of industries means that demand will increase significantly and in order to provide sufficient macroalgal biomass on the potential scale required for economically viable industries, large scale cultivation in coastal waters is required (Schlarb-Ridley and Parker, 2013; Radulovich et al., 2015; Lehahn et al., 2016; Buschmann et al., 2017). Europe's extensive coastline comprises huge potential to contribute significantly to the supply of macroalgal biomass primarily due to the wide availability of numerous coastal sites to cultivate macroalgae.

Site suitability for algal cultivation can be influenced by several factors e.g. available area, photosynthetically active radiation (PAR), nutrient load, salinity and water motion (Kerrison et al., 2015; Wood et al., 2017; van der Molen et al., 2018). A further key issue in selecting sites for macroalgal farms at sea is the potential for genetic interaction of cultivated specimens on longline systems with wild populations via gamete or spore dispersal (Stévant et al., 2017). Understanding the distance over which gene flow can occur is thus vital to understand the potential for macroalgal cultivation to impact adjacent ecosystems (Coleman et al., 2009; Luttikhuizen et al., 2018). Propagule gametes and spores have a planktonic phase with the ability to swim and actively seek out optimal settlement substrata (Fredriksen

et al., 1995), and photosynthesis can extend their viability in the water columns and thus their dispersal potential (Reed et al., 1992). In addition, spores may survive in the water column for longer periods of time following periods of dormancy (Schiel and Foster, 2006), and while there is little evidence to suggest this occurs in the field, it is a condition which is exploited in cultivation hatcheries (Edwards and Dring, 2011).

Although settlement behaviour and biological traits such as buoyancy and sinking rates play a role in dispersal at the microscale (cm – mm) near the substrate (Stevens et al. 2008), water motion – predominantly currents – and where in the water column the spore is (near the surface or near the substrate) strongly regulates transport and dispersal of propagules with limited inherent mobility, and will influence macroalgal gene flow (Norton, 1992; Gaylord et al., 2002). While historically it was thought that gene flow in macroalgae was very limited by geographical distance (Billot et al., 2003), more recent studies suggest that effective dispersal of gametes may extend to up to several kilometres (Couceiro et al., 2013, Brennan et al., 2014) and even up to 200km for *Laminaria hyperborea* (Fredriksen et al., 1995). This is perhaps unsurprising considering that many coastal regions experience current velocities > 0.5 m s^{-1} suggesting that currents play a significant role in the transport of propagules. Although small-scale macroalgal cultivation takes place in sheltered areas e.g. in the United Kingdom (Strangford Lough in Northern Ireland and Oban in Scotland), Pleubian in Brittany, France and Ventry Harbour in Co. Kerry, Ireland, the implications of potentially extensive large-scale cultivation developed in coastal regions are still unknown. Interest in cultivation is growing with farms now being developed in the USA and Canada (Breton et al., 2018). Annual harvesting of farmed strains will mean that cultivated adult populations will be transient, but even so, harvesting is unlikely to be 100% efficient, and some cultivated material can also become fertile early in the season (Parke, 1948), so that off-shore

macroalgal farms may lead to potential genetic mixing of cultivated and indigenous populations.

One target species for European cultivation is the sugar kelp, *Saccharina latissima* (previously *Laminaria saccharina*). The genus *Saccharina* is widely distributed across both the Northern and Southern Hemispheres (Bolton, 2010), with Bartsch et al. (2008) highlighting it as a major Pacific-Atlantic species complex, while *S. latissima* is widespread in the North Atlantic (Luttikhuizen et al., 2018), reaching as far north as the Arctic and as far south as Portugal and New York (Lee and Brinkhuis, 1986; Smale et al., 2013; Guzinski et al., 2016). Like all kelps, *S. latissima* has a biphasic life history, with an alternation of generations (Dayton, 1985; Schiel and Foster, 2006). Fertilisation generally tends to happen over short distances (Dayton, 1985). The reproductive success of kelp thus depends on availability of female gametes and their proximity to male gametes.

Given the importance of understanding both local and regional patterns of gene flow to the conservation and conscientious management of commercial areas, the aim of the present study was to identify the factors that determine patterns of genetic variation of *S. latissima* populations in the Irish Sea. We used a combination of microsatellite genotyping and hydrodynamic modelling to test the connectivity of wild populations, thus, providing valuable information on the optimal locations of future coastal kelp cultivation farms and the sourcing of wild material suitable for cultivation.

2. Materials and methods

2.1. Sampling and DNA extraction

To characterise native wild populations of *S. latissima*, individual samples were taken from sites chosen to be representative of conditions across the Irish and Inner Seas off the West Coast of Scotland. Each site was separated by distances > 10 km to test whether (and how) hydrodynamic conditions determine connectivity and how far these algal spores travel to understand potential genetic interaction of cultivated kelp on natural populations. Nearshore cultivation takes place in Strangford Lough (Queen's University Belfast [QUB] experimental site) and, consequently, samples were taken from inside the lough, as well as from around the Northern Ireland coastline, the Isle of Man, and the west coast of Scotland (Fig. 1). In total, 14 sites were sampled (eleven from Northern Ireland, two from Scotland and one from the Isle of Man, Table 1, Fig. 1). At each site, thirty mature (>1m) *S. latissima* sporophytes were randomly selected from discrete populations and a small disc 0.7cm in diameter was hole-punched approximately 4cm from the stipe/laminar junction in an area free of epiphytes and stored in powdered silica gel. DNA was extracted using the CTAB method (Doyle and Doyle 1987).

2.2. Microsatellite genotyping

Five species-specific microsatellite loci were developed using a modified version of the biotin / streptavidin capture method originally outlined by Kijas et al. (1994). Primer sequences are given in Table 2. A further locus (Zspj39) originally developed for *S. japonica* (Zhang et al. 2014) was also used. Loci Zspj8 and Zspj40 from the same study were tested, but could not be consistently amplified and so were not used. PCR was carried out in a total volume of 10 µl containing 100 ng genomic DNA, 5 pmol of 6-FAM-, ROX- or HEX-

labelled M13 primer, 0.5 pmol of M13-tailed forward primer, 5 pmol reverse primer, 1x PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA, USA). PCR was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following conditions: initial denaturation at 94 °C for 3 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system. (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored using the GENEMAPPER software package (v4.1; Applied Biosystems) using LIZ-500 size standards, and were checked by comparison with previously sized control samples. Chromatograms were all inspected visually.

2.3. Genetic data analysis

GENEPOP (V3.4; Raymond and Rousset, 1995) was used to test for linkage disequilibrium between nuclear microsatellite loci. To estimate genetic diversity within sites, levels of observed (H_O) and expected (H_E) heterozygosity, levels of allelic richness (A_R) and fixation indices (F_{IS}) were calculated using the FSTAT software package (V2.9.3.2; Goudet, 2001). Significance of F_{IS} was determined by 10,000 randomisation steps. The overall level of genetic differentiation between sites was estimated using Φ_{ST}, which gives an analogue of F_{ST} (Weir and Cockerham, 1984) calculated within the analysis of molecular variance (AMOVA) framework (Excoffier et al., 1992) using the ARLEQUIN software package (V3.5.1.2; Excoffier and Lischer, 2010). To further identify possible patterns of genetic structure, the software package BAPS (V5; Corander et al., 2003) was used to identify clusters of genetically similar sites using a Bayesian approach. Ten replicates were run for all possible values of the maximum number of clusters (K) up to $K = 14$, the number of sites sampled, with a burn-in period of 10,000 iterations followed by 100,000 iterations. An AMOVA was also carried out

based on the groups delineated by the BAPS analysis (see Results), and pairwise Φ_{ST} values between sites were also estimated.

A test for isolation-by-distance **(**IBD; Rousset 1997) was carried out to test the null hypothesis of a stepping-stone model of gene flow between sites. The ISOLDE test implemented in the GENEPOP software package was used to assess the relationship between genetic distance, measured as $F_{ST}/(1-F_{ST})$, and geographical distance between pairs of sites, measured as the shortest distance across water. 1,000 permutations were used for the Mantel test.

To test the power of the six microsatellites used in the study to detect low levels of population genetic differentiation, simulations were carried out using the POWSIM software package (V4.0; Ryman and Palm, 2006). Simulations were carried out for an effective population size of N_e = 1,000 to yield F_{ST} values of 0.0005 – 0.0050. In all cases, 1,000 replicates were run and the power of the analysis was indicated by the proportion of tests that were significant at $P < 0.05$ using the observed allele frequencies for the six microsatellite loci (for $F_{ST} = 0$ this corresponds to the Type I [α] error).

2.4. Hydrodynamic and particle tracking modelling

Dispersal of *S. latissima* spores was numerically predicted using a particle tracking module coupled to the Irish Sea Hydrodynamic Model (Elsäßer et al., 2010) using MIKE21 modelling software (DHI Water and Environment software package: www.dhisoftware.com). To determine the current field, the Irish Sea Model uses a finite volume method by solving a depth averaged shallow water approximation. While fertile *S. latissima* material can be found between April and November (K. Mooney, Personal Observation), current flow is tidally driven, which is predictable and varies little throughout the year, therefore the model

was run for the month of September as a representative period incorporating two neaps and two spring tides.

Two significant temporal factors that will affect the distance that spores travel are tidal state (e.g. flood, ebb or slack tide), which determines how spores are released into the water column, and the length of time that spores are viable. Currently, the optimal tidal state for *S. latissima* spore release is unknown, therefore, a trickle release approach was adopted whereby 200 particles (a proxy for spores) were released every 5 min during the simulation. While there are specific studies on the length of time that spores are viable, these are laboratory based observations with the general consensus that free floating spores are short lived and do not remain viable for more than a few days (Suto, 1950; Kain, 1964; Jones and Babb, 1968; Reed et al., 1992). While propagules of the green alga *Ulva* are capable of living for up to 8 days (Jones and Babb, 1968), studies of the giant kelp, *Macrocystis pyrifera* concluded that no zoospores could swim for longer than 120 h and, in the dark, none longer than 72 h (Reed et al., 1992). Kain (1964) reported that zoospores of another North Atlantic kelp, *Laminaria hyperborea,* could not swim longer than 20 h. Based on the information from the studies on the kelps, particles (spores) were given a life span of 5 days in the model before they were terminated. To reach a pseudo stationary pattern, a time series analysis was carried out in the second to last week of the simulation where at each site the number of particles (spores) on the bed per square metre was derived in relation to the number released in the model to this point. This derived the average concentration of particles reaching the bed per square metre.

Two important considerations when simulating transport processes in the marine environment are advection and dispersion, where advection is the mean flow ($\bar{u}$: derived from the hydrodynamic model) that transports particles from one location to another and dispersion is driven by factors such as non-resolved turbulence or eddies. Horizontal and vertical

dispersal movement of the particles were resolved using the Langevin equation. For horizonal movement, the scaled eddy viscosity was used and in the absence of any dispersion information and the recommended constant value in the software of 1.0 was used. For the vertical dispersion, a constant dispersion value of 0.01 m^2/s was used. As flow velocity changes with depth, a logarithmic velocity profile was calculated based on the bed friction velocity, a parameter calculated in the hydrodynamic model. Each site was simulated separately with particles released 0.5 m off the substratum to represent an approximate height of *S. latissima* kelp canopy.

3. Results

3.1. Population genetic analysis

No significant evidence of consistent linkage disequilibrium (i.e. involving the same loci) was detected between any of the six nuclear microsatellites analysed (4 out of 210 tests). Between six (Zspj39) and 61 (Sac-1H08) alleles were detected per locus, with a total of 133 (mean = 21.167 per locus; Table 2). F_{IS} values by locus ranged from -0.053 (Zspj39) to 0.211 (Sac-1F02). Within sites, levels of allelic richness (A_R) averaged over loci ranged from 5.546 (S11 – St John's Point) to 7.144 (S4 – Knockinelder), with a mean value of 6.261 (Table 1). Levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.554 (S12 – Port Erin) to 0.826 (S11 – St John's Point; mean = 0.635), and from 0.616 (S6 – Marlfield) to 0.776 (S1 – Rathlin Island; mean = 0.666) respectively. Heterozygote deficits measured as F_{IS} values were significantly different from zero in seven of the 14 sites studied, ranging from -0.048 (S7 – Walter Shore) to 0.165 (S4 – Knockinelder; mean = 0.074).

The AMOVA indicated that 5.89% of the total genetic variation was partitioned between sites (Φ_{ST} = 0.059; $P < 0.0001$; Table 3). The BAPS analysis identified four genetic clusters: the first contained the Northern Ireland sites north of Belfast Lough (Rathlin Island and Carnlough), the second comprised all the Northern Ireland sites south of Belfast Lough (Bangor, Knockinelder, Tara Bay, Marlfield, Walter Shore, The Dorn, Kircubbin, Audleystown Rocks and St John's Point), the Port Erin site from the Isle of Man formed the third, and the two Scottish sites (Stranraer and Troon) made up the final group (Fig. 2). Multiple independent runs always gave the same outcome. The group-level AMOVA indicated that 7.82% of the total genetic variation was partitioned between the four groups identified by the BAPS analysis (Φ_{CT} = 0.0782; $P < 0.0001$; Table 3). Finally, a significant pattern of isolation-by-distance was observed across all sites analysed, but not among the

group of sites south of Belfast Lough alone (Fig. 3). Population-pairwise Φ_{ST} values were significant in all but 13 of the 91 comparisons, which were all between populations from the Southern group, and ranged from 0.009 (S7 – Walter Shore vs. S9 – Kircubbin) to 0.171 (S11 – St John's Point vs. S12 – Port Erin; Supplementary Table S1).

The simulation studies suggested that the microsatellite data were able to detect F_{ST} values of as low as 0.0030 95% of the time, and in all simulations for F_{ST} values of 0.0045 and above (Fig. S1).

3.2. Hydrodynamic and particle tracking modelling

The most notable feature from the numerical simulations of the particle tracking modelling is the minimal exchange of particles between Northern Ireland, Scotland and Isle of Man populations (Fig. 4). Greatest dispersal was observed at Carnlough (S2) along the north coast of Northern Ireland where tidal flows can reach up to approximately 1 m/s. The simulations also clearly showed overlap between this site and Rathlin Island (S1). Port Erin (S12), at the tip of the Isle of Man, is also a high flow area with dispersion quite prominent around part of the island, but no overlap between any of the other locations is observed. Given that spores had a lifespan of five days and were released continuously throughout the month of September, localised particle retention was observed at each site, particularly at Bangor (S3). Localised retention coincides with associated low-flow regimes such as at Bangor (S3), the entrance to the Belfast Lough Harbour, and Troon (S14) in Scotland. Conversely, sites inside and adjacent to Strangford Lough (Sites 4-11) appeared to be well-connected, with dispersal in both directions through the entrance to the Lough observed from The Dorn (S8) and St. John's Point (S11).

4. Discussion

These findings reveal a hierarchical structure of genetic differentiation in the kelp *Saccharina latissima* across the Irish and Inner Seas. At smaller scales (less than a few 10s of km), gene flow appears to be fairly extensive, with evidence of connectivity between sites due to local currents. At larger scales, however, factors such as freshwater efflux and open water would appear to represent barriers to gene flow. Together, these patterns suggest that factors other than simple geographical distance and proximity need to be taken into account when planning the siting of kelp farms and sourcing culture broodstock, with the aim of minimizing gene flow to and from natural populations.

The overall value for population differentiation observed in the present study (Φ_{ST} = 0.059) was lower than both the mean (0.211) and median (0.130) values reported for 101 macroalgal studies by Durrant et al. (2014), and for the subset of these studies that examined kelps (mean = 0.148; median = 0.080; n = 21). While macroalgae typically display isolation-by-distance (IBD), our findings suggest that for *S. latissima*, this may be more pertinent at larger geographic scales, and they also reflect the results of previous genetic studies on the species, which found greater levels of differentiation at geographical scales over an order of magnitude greater than those examined in the present study. Nielsen et al. (2016) observed a main pairwise F_{ST} of 0.096 between populations from a transition zone across the North and Baltic seas, with some evidence of IBD. It should be noted, though, that this was partly due to differences between marine and brackish populations (Φ_{ST} = 0.127), whilst lower mean values were observed between pairs of populations classed as either marine (F_{ST} = 0.073) or brackish (F_{ST} = 0.040). Guzinski et al. (2016) observed a mean value of F_{ST} = 0.358 between European populations spanning several thousand kilometres, but with no clear evidence of IBD, whilst Luttikhuizen et al. (2018) estimated F_{ST} = 0.267 between natural populations

ranging from Northern Norway to Brittany, a range around an order of magnitude greater than that covered by the present study.

The isolation of Scottish and Isle of Man populations from those in Northern Ireland and from each other was expected, given the current flow velocity and direction and large distances that spores would have to cross to breed similar populations, although the separation of the Northern Irish populations into separate northern and southern groups was unexpected. The Scottish populations are *ca*. 65 km apart, with Stranraer (S13) experiencing current flows up to 0.4 m/s at the south of Loch Ryan while Troon (S14) is on the edge of the Firth of Clyde experiencing flows <0.1 m/s. Despite the large geographical distance and low flow velocities, these sites belong to the same genetic cluster. By contrast, the Northern Irish sites cover a total distance of *ca.* 140 km from Rathlin Island (S1) in the north to St. John's Point (S11) at the southernmost site. This coastline is a combination of wave and current conditions with low flow velocities < 0.1 m/s in the loughs (Strangford Lough and Belfast Lough), and wave sheltered harbour (where the Rathlin samples were taken). Microsatellite analysis showed that these populations form two distinct genetic clusters. The northern pair which form the first cluster, Rathlin Island (S1) and Carnlough (S2), are *ca.* 40 km apart, while the southern group included sites along the outside of the Ards peninsula (Bangor (S3) and St John's Point (S11), *ca.* 65 km apart), as well as those from the predominantly current landlocked Strangford Lough (Kregting and Elsäßer 2014). While geographical distances between sites within these two clusters are similar to that between the Scottish populations, it should be noted that the sites within Strangford Lough are genetically similar to those outside the Lough, despite the marked habitat discontinuities. This shows that there are factors other than geographical distance or habitat discontinuity affecting genetic differentiation patterns, which could reflect the proportion (64%) of significant pairwise Φ_{ST} values between these sites and the small but significant level of differentiation between populations within the four

clusters (1.55% of the total genetic variation), although there were no apparent geographical patterns in these values. Similar genetic differentiation was found in eastern Maine, USA, where two populations which were ~50km apart were genetically different while nearby populations up to 90km apart had genetic mixing (Breton et al., 2018).

Hydrodynamic modelling shows patterns of spore dispersal that are generally consistent with the genetic patterns seen in the Northern Irish sites sampled. Simulated spore release from the Rathlin Island (S1) and Carnlough (S2) sites show that there is mixing between both sites. Simulated releases from The Dorn (S8) (within Strangford Lough) and St. John's Point (S11) (outside the Lough) show mixing of spores and explain how the habitat discontinuity within the southern group can be overcome by hydrodynamic influence. Populations of *S. latissima* in and around Strangford Lough exhibit a higher degree of connectivity than populations of *Laminaria digitata* from the same area (Brennan et al. 2014). The lack of significant IBD in the southern populations in the present study is reflected in the low levels of F_{IS}, suggesting substantial gene flow and little inbreeding, and is in contrast to significant levels of both IBD and inbreeding observed in *L. digitata* in the previous study. Interestingly, at the north of the southern group, spores released from Bangor (S3) apparently have a very narrow dispersal while those from Carnlough (S2) in the northern group, although they have a very wide dispersal, do not appear to travel far enough to reach the Bangor sites. These sites are situated either side of Belfast Lough, which has a large freshwater plume that enters the Irish Sea that results in fluctuating salinity (Service et al., 1996). It is most likely this change in salinity gradient represents a barrier to spore dispersal and survival.

With the projected increase in coastal kelp farming, it is important to be aware of the potential for interaction between farmed and natural populations, especially given the capacities for dispersal indicated in the present study. Although lack of suitable (i.e. rocky) substrate has been proposed to be a major dispersal barrier for several kelps (Alberto et al.,

2010; Couceiro et al., 2013; Robuchon et al., 2014), the establishment of longline systems and similar growth structures may provide a way to overcome both substrate and isolation-by-distance barriers, by acting as "stepping stones" for dispersal of fertile material and gametes. However, kelp gamete dispersal does not necessarily lead to successful reproduction and colonisation. Even if spores are dispersed, there is a "Goldilocks-like" cocktail of criteria to be met for success, including a sufficient quantity of male and female gametes for fertilisation to occur, suitable substrate for gametophytes and juvenile sporophytes to settle on, low enough grazing pressure, suitable photosynthetically active radiation (PAR) and temperature, sufficient nutrient and salinity gradients, and the right amount of current and wave exposure (Gaylord et al., 2002; Schiel and Foster, 2006; Andersen et al., 2013, Kregting et al., 2015; van der Molen et al., 2018). For offshore sites, it is highly unlikely that such a combination of factors will be met although the potential is greater in nearshore sites.

The direct transplant of seaweed cultures from one region to another could overcome natural obstacles to gene flow and dispersal such as hydrodynamic or salinity barriers, allowing establishment of genetically distinct populations in a new region. Translocation of seaweed species into non-native areas has led to establishment of invasive species, such as the spread of *Undaria pinnitifida* from cultivated sites in Brittany, France to Portugal, the Netherlands, Belgium, Ireland and the UK (Kraan, 2016). Consequently, new marine policy guidance from the Scottish Government recommends that only species local to the production area should be farmed, to reduce the risk of establishment of invasive species in the future (Marine Scotland, 2017). In addition, poleward shifts of several kelp species are evident, for example species with a warmer water affinity, such as *Laminaria ochroleuca,* are expanding their ranges while colder water species, such as *Alaria esculenta*, *L. hyperborea* and *L. digtata* are predicted to contract (Smale et al., 2015; Brodie et al. 2014; Franco et al. 2018).

The presence of kelp farms may exacerbate these species range shifts, by extending the typical dispersal potential of farmed kelp spores via cultivation sites acting as stepping stones for settlement of wild kelp and fertile farmed adults. Uncertainties associated with these anthropogenic translocations of genetically differentiated material means that great care will need to be taken to minimise potential modifications of natural dispersal processes and resulting patterns of genetic diversity following the establishment of kelp farms.

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Table 1

Details of populations studied. N – number of individuals analysed; A_R – allelic richness; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{IS} – inbreeding coefficient (* $P < 0.05$, ** $P < 0.01$, NS Non-significant).

Site	Name	Lat (N)	Long (W)	N	A_R	H_O	H_E	F_{IS}
1	Rathlin Island	55.293	6.194	29	6.038	0.781	0.776	-0.006^{NS}
2	Carnlough	55.004	5.983	30	6.793	0.595	0.674	0.118**
3	Bangor	54.666	5.665	29	6.193	0.595	0.695	0.145**
4	Knockinelder	54.383	5.482	28	7.144	0.594	0.709	0.165***
5	Tara Bay	54.350	5.490	29	6.250	0.638	0.673	0.052^{NS}
6	Marlfield	54.402	5.580	30	6.340	0.583	0.616	0.054^{NS}
7	Walter Shore	54.380	5.558	31	5.790	0.660	0.631	-0.048^{NS}
8	The Dorn	54.440	5.548	30	6.423	0.618	0.640	0.035^{NS}
9	Kircubbin	54.485	5.538	30	6.297	0.620	0.650	0.048^{NS}
10	Audleystown Rocks	54.380	5.572	30	5.793	0.618	0.646	0.044^{NS}
11	St John’s Point	54.227	5.660	23	5.546	0.826	0.644	0.109*
12	Port Erin	54.089	4.763	17	6.201	0.554	0.642	0.141**
13	Stranraer	55.008	5.048	27	6.488	0.646	0.704	0.084*
14	Troon	55.541	4.670	14	6.356	0.565	0.625	0.099*

Table 2

Details of microsatellite markers used in this study. Zspj39 was originally developed for *Saccharina japonica* (Zhang *et al.* 2014). * F_{IS} value for locus Sac-1F02 significant ($P = 0.04$).

Locus	Repeat	Primers (5'-3')	Alleles	F_{IS}	Size range (bp)
Sac-1B02	$(TTG)_{20}$	AGCCCTCTCTCAAGTCGTGCGT	28	0.029	190-280
		TCTCCGCACAAGCCGTTATCCC			
Sac-1B05	$(TGC)_8$ … $(TGC)_5$ … $(TGC)_7$	TGCGGTAGCGGTAGCACTTTGA	11	-0.053	233-278
		GCGTGTACCCCGAAATCGGACA			
Sac-1F02	$(GCT)_7(GCC)_2(GCT)_3GCC(GCT)_5(GAT)_4$	TACGAGGAGGGCGTGCTGGTTT	10	0.211*	177-273
		GTGCTGTATTTACGCGATCTCGTGGG			
Sac-1H08	$(TTGT)_{16}$	TAATGTCTCTTTTATGCATGCC	61	0.075	302-614
		GGTGTGGCTGTCGGACCC			
Sac-2C10	$(CAG)_{10}$	ATCAAACACAACTGTTGCTGGAATGGA	17	0.015	334-382
		GCACTGCCTTGGAAGAGCGGAA			
Zspj39	$(GGA)_{12}$	CTCGGTTCAAAGTTCCGCAAG	6	0.042	243-264
		CATCCGCAATTTCTTCCACGG			

Table 3

Analysis of Molecular Variance (AMOVA). The groups in the lower Table correspond to the four genetic clusters delineated by the BAPS analysis.

Source of variation	df	Sum of squares	Variance components	% variation	*P*
Among populations	13	100.142	0.111	5.89	$P < 0.001$
Within populations	736	1305.242	1.773	94.11	

Source of variation	df	Sum of squares	Variance components	% variation	*P*
Among groups	3	65.583	0.153	7.82	$P < 0.001$
Among populations within groups	10	34.559	0.030	1.55	$P < 0.001$
Within populations	736	1305.242	1.773	90.63	$P < 0.001$

Figure Legends

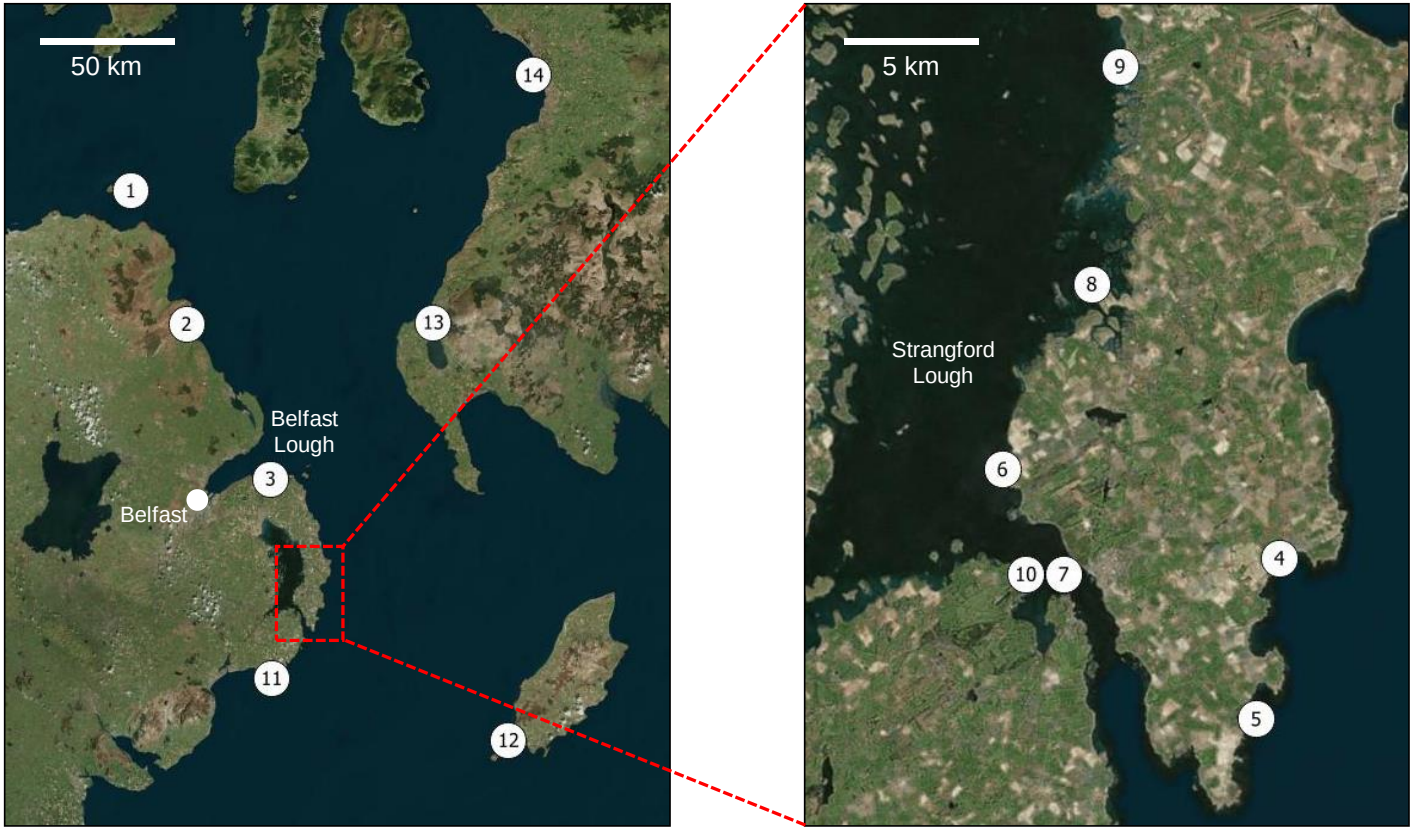
Fig. 1. Map of sampling sites – inset shows detail of populations in and around Strangford Lough. Numbers refer to Table 1.

Fig. 2. Results of the BAPS analysis. Different colours represent assignment to one of four different genetic clusters. Numbers refer to populations in Table 1 and Figure 1.

Fig. 3. Results of the isolation-by-distance analysis: (a) all populations; (b) populations from the southern (i.e. yellow) BAPS cluster.

Fig. 4. Particle distribution from eight representative release sites (1, 2, 3, 8, 11, 12, 13 and 14; Table 1 and Figure 1). Release of particles is constant simulating trickle spawning; time step 5 min, 200 particles per time step (see Materials and Methods for details).

Fig. S1. Results of the POWSIM analysis. The Y-axis represents the power of the markers to successfully recover the value of F_{ST} indicated on the X-axis, expressed as the proportion of 1000 simulations (see text for details). For $F_{ST} = 0$, this is the Type I (α) value.


50 km
5 km
Belfast
Lough
Belfast
Strangford
Lough
1
2
3
4
5
6
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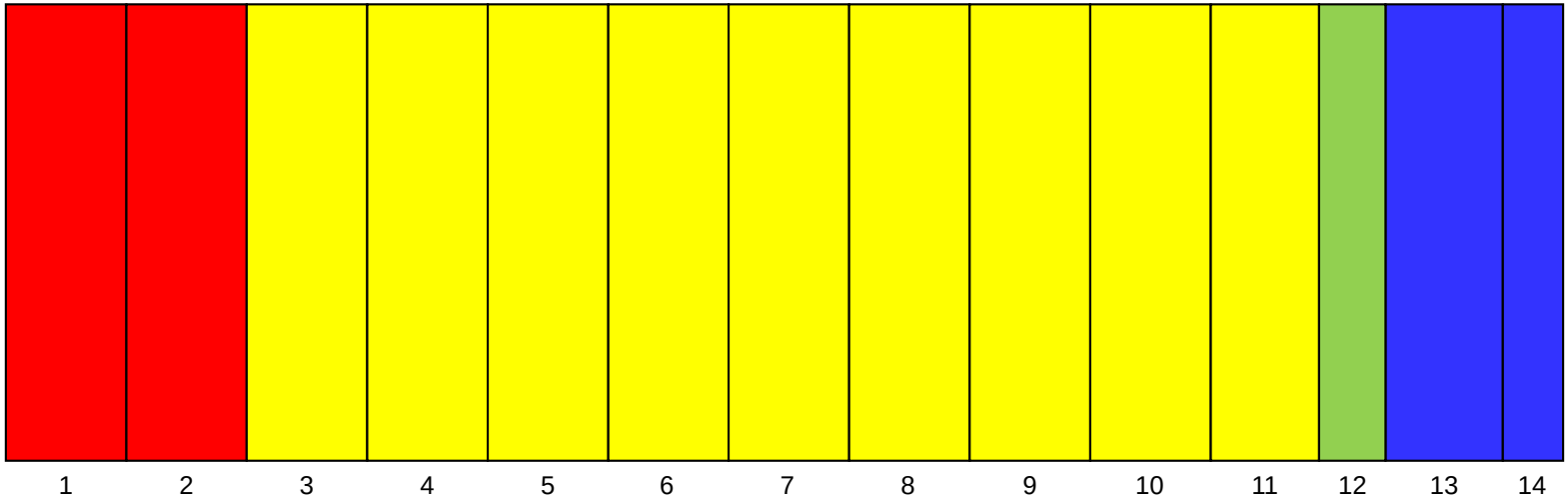
9
10
11
12
13
14


1
2
3
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5
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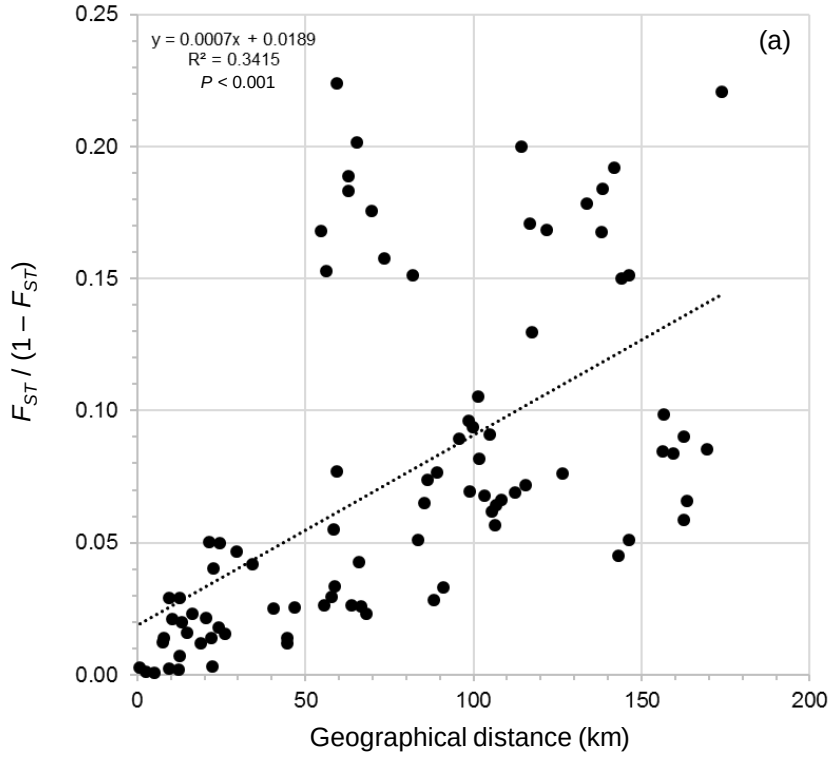
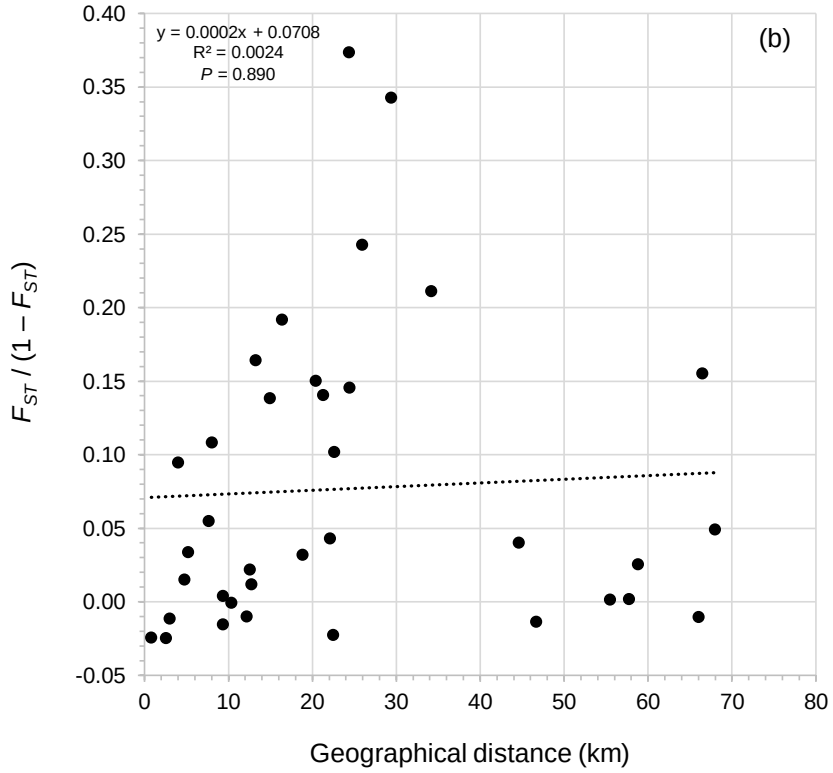
14


y = 0.0007x + 0.0189
R² = 0.3415
P < 0.001
(a)
0.25
0.20
0.15
0.10
0.05
0.00
0
50
100
150
200
F_{ST} / (1 – F_{ST})
Geographical distance (km)


y = 0.0002x + 0.0708
R² = 0.0024
P = 0.890
(b)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
10
20
30
40
50
60
70
80
F_{ST} / (1 – F_{ST})
Geographical distance (km)

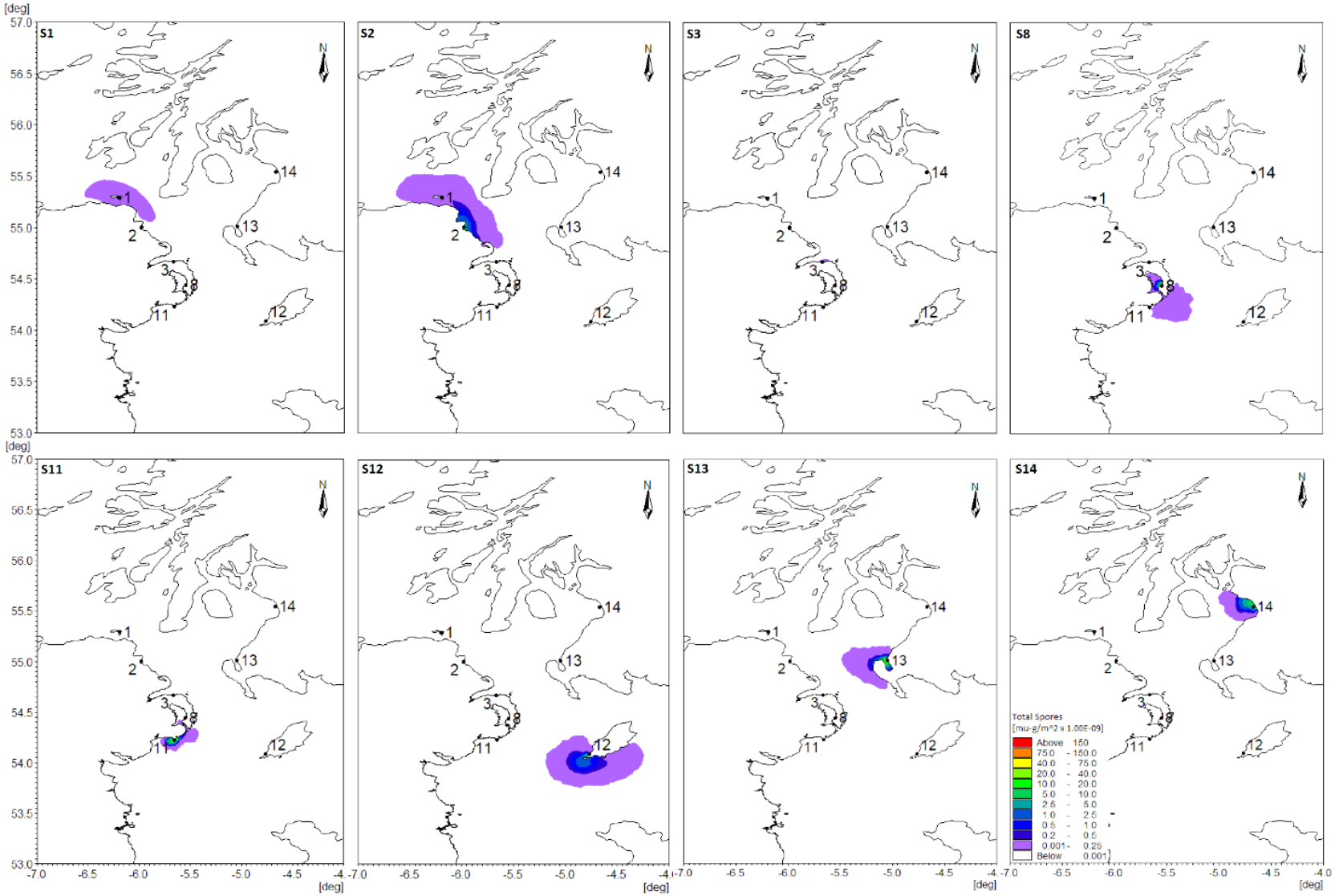
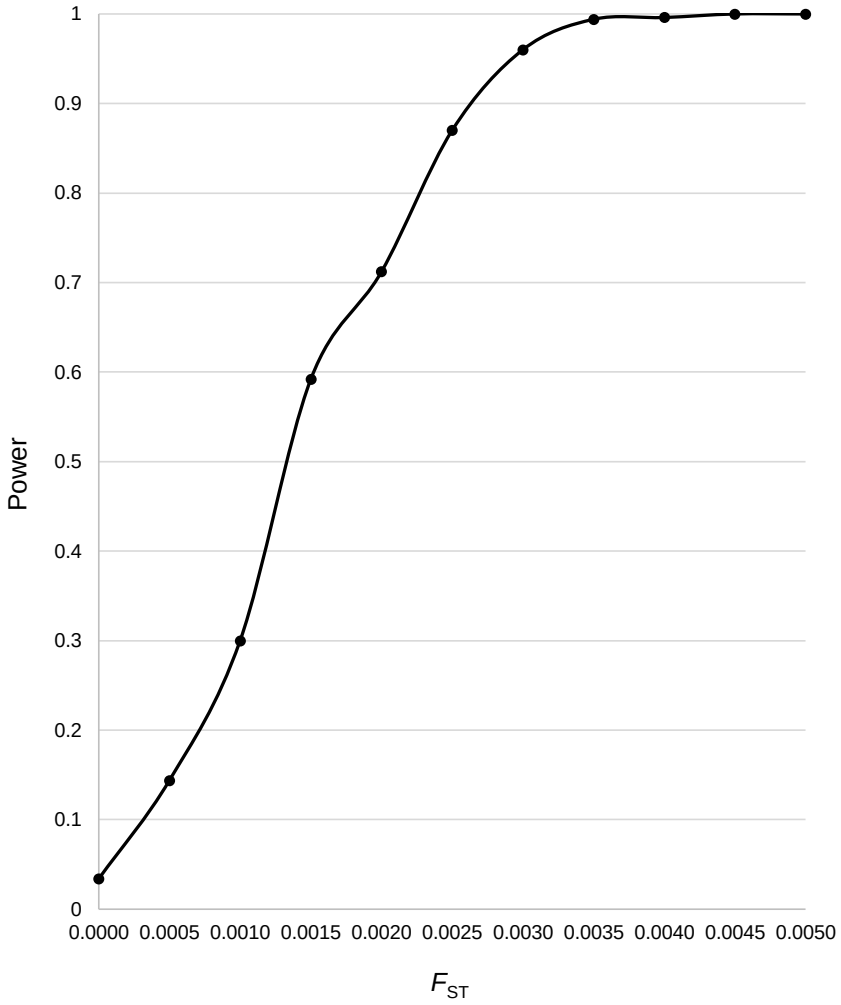

S1
S2
S3
S8
S11
S12
S13
S14
N
[deg]
Total Spores
[mu-g/m^2 x 1.00E-09]
Above 150
75.0 - 150.0
40.0 - 75.0
20.0 - 40.0
10.0 - 20.0
5.0 - 10.0
2.5 - 5.0
1.0 - 2.5
0.5 - 1.0
0.2 - 0.5
0.001 - 0.25
Below 0.001

Table S1 Population-pairwise Φ_{ST} values. Site numbers refer to those in Table 1. Values not significantly different from zero are given in italics.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-													
2	0.025	-												
3	0.061	0.010	-											
4	0.114	0.048	*0.006*	-										
5	0.149	0.069	0.028	*-0.009*	-									
6	0.158	0.086	0.036	0.019	0.029	-								
7	0.154	0.083	0.027	0.018	0.027	*-0.001*	-							
8	0.132	0.058	0.027	0.014	0.021	*0.003*	0.015	-						
9	0.133	0.063	0.025	0.014	0.014	*0.005*	0.009	*0.000*	-					
10	0.142	0.083	0.018	0.015	*0.007*	*-0.008*	*-0.003*	*0.005*	*-0.004*	-				
11	0.157	0.076	0.023	*0.002*	*-0.008*	0.035	0.040	0.035	0.031	0.040	-			
12	0.088	0.075	0.073	0.123	0.141	0.169	0.154	0.151	0.137	0.152	0.171	-		
13	0.135	0.075	0.054	0.028	0.030	0.067	0.079	0.063	0.069	0.062	0.047	0.147	-	
14	0.167	0.094	0.061	0.045	0.038	0.070	0.086	0.058	0.076	0.079	0.060	0.174	0.024	-


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Power
0.0000 0.0005 0.0010 0.0015 0.0020 0.0025 0.0030 0.0035 0.0040 0.0045 0.0050
F_{ST}